IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Wolfgang Christian HANS
Lothar STEIDLER
Erik René REMAUT

Serial No.: **10/030,390**

Filing Date: **April 16, 2002**

For: **THE DELIVERY OF TREFOIL PEPTIDES**

§ Art Group No.: **1645**

§ Examiner: **Sarvamangala Devi**

§ Confirmation No.: **2253**

§ Atty. Dkt.: **13475.0002.PCUS00**

RULE 132 DECLARATION OF LOTHAR STEIDLER

I, Lothar Steidler, of The Alimentary Pharmabiotic Centre, BioSciences Building, University College Cork, Cork, Ireland, hereby declare as follows:

1. I am employed by University College Cork, Cork, Ireland.

2. I am a guest Professor at the University of Ghent, Belgium, associated with the Department of Biology.

3. I received a Ph.D. degree in Biotechnology from Ghent University in Belgium.

4. I am a co-inventor of the invention claimed in the above captioned Patent Application.

5. I have reviewed the Office Action dated July 15, 2005, for the above captioned application, in which the Examiner rejected the claims as being unpatentable over Podolsky (US 6,221,840) in view of Le Page *et al.* (US 6,221,648) or Steidler *et al.* (US 6,605,286), Wells *et al.* (Mol. Microbiol. 8:1155-1162, June 1993) and Tran *et al.* (Gut 44:636-642, May 1999), and further in view of Silk (WO 82/03329).

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6. I am a co-author of Vandenbroucke *et al.*, Gastroenterology 127(2): 502-13; 2004 (hereafter "Vandenbroucke *et al.*"), a copy of which is enclosed for the Examiner's consideration.

7. I supervised the following experiments that were described in Vandenbroucke *et al.* These experiments provide comparative data revealing that bacterial delivery of trefoil factor (TFF) peptides is considerably more efficacious than oral administration of much larger quantities of purified TFF peptides.

Experiment 1: Protective Effect of Murine TFF (mTFF)-Secreting *L. lactis* Against DSS-Induced Acute Colitis

Acute colitis was induced in mice by administration of 5% DSS in the drinking water. During administration of DSS, 4 groups of mice received daily inoculation of 2 X 10^9 CFU of *LL-mTFF1*, *LL-mTFF2*, *LL-mTFF3*, or *LL-pTREX1* (vector control) through an intragastric catheter. LL-mTFF1, LL-mTFF2 and LL-mTFF3 are representative of a recombinant microorganism expressing a trefoil peptide as recited in claim 1 of the above captioned Patent Application. On day 8, the mice were killed and examined for myeloperoxidase (MPO) activity in the colon and histologic analysis of the middle colon.

The results show that the groups treated with LL-mTFF1, LL-mTFF2 and LL-mTFF3 had significantly lower levels of MPO (> 60% lower, $P < 0.001$) than the LL-pTREX1-treated group (*see* Figure 3C of Vandenbroucke *et al.*) The results also show that treatment with LL-mTFF reduced epithelial damage and inflammatory infiltration in acute colitis by 50% (*see* Figure 3D and Figures 4D-F of Vandenbroucke *et al.*) These results suggest that daily

intragastric inoculation of mice with doses of 2 X 10^9 CFU of LL-mTFF1, LL-mTFF2, or LL-mTFF3 had a substantial protective effect against all aspects of acute DSS-induced colitis.

Experiment 2: Therapeutic Effect of mTFF-Secreting *L. lactis* Against Established Acute DSS-Induced Colitis

Acute DSS colitis was induced in mice as described above. With the return of normal drinking water after 7 days, treatment with various LL-mTFF was initiated and maintained for 5 days. One day after the last LL-mTFF inoculation, the mice were killed and examined for MPO activity in the colon and histologic analysis of the middle colon.

The results show that treatment with LL-mTFF reduced accumulation of MPO in the DSS-induced inflamed colon by 30% (*see* Figure 3E of Vandenbroucke *et al.*) The LL-mTFF1, LL-mTFF2 and LL-mTFF3 groups exhibited significantly lower levels of MPO ($p < 0.05$) compared with the LL-pTREX1-treated group. The results also show that treatment with LL-mTFF reduced epithelial damage and lymphoid infiltration in acute colitis by at least 30% (*see* Figure 3F of Vandenbroucke *et al.*) These results suggest that daily intragastric inoculation of mice with 2 X 10^9 CFU of LL-mTFF1, LL-mTFF2, or LL-mTFF3 for 5 days leads to acceleration of healing process of established acute colitis.

Experiment 3: Efficacy of Purified TFF in Prevention or Treatment of Acute Colitis

To compare the efficacy of oral administration of purified TFF peptides with LL-mTFF delivery, purified mTFF peptides were given orally during or after administration of DSS using the same treatment regimes as those used for LL-mTFF treatment. The results show that during administration of DSS, oral treatment with 5 μg or 50 μg purified mTFF1 daily did not decrease

the MPO levels in the inflamed colon (*see* Figure 3C of Vandenbroucke *et al.*) and did not improve the histologic score of the middle colon (*see* Figure 3D and Figures 4I-J of Vandenbroucke *et al.*) Daily oral administration of 5 μg or 50 μg of purified mTFF1 for 5 days after the DSS challenge gave similar results (*see* Figure 3E and 3F of Vandenbroucke *et al.*)

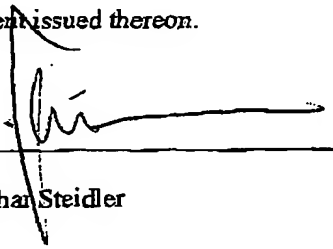
The above experiments demonstrate a superior efficacy of bacterial TFF1 delivery when compared with direct oral administration of purified TFF1, as clearly suggested by the below table. The table summarizes the aspects of *LL-mTFF* and *purified mTFF* treatments that are described above.

Factors Compared	**LL-mTFF1**	**Purified mTFF1**
Amount of peptide delivered (ng)	32	5000 or 50,000
Animal model treated	DSS-induced mice	DSS-induced mice
Type of treatment	Intragastric inoculation	Intragastric inoculation
Treatment regime	Daily during DSS challenge; or Daily for 5 days after DSS challenge	Daily during DSS challenge; or Daily for 5 days after DSS challenge
Results (during DSS challenge)	> 60% lower levels of MPO; Reduced epithelial damage and inflammatory infiltration in the middle colon by 50%	Neither decrease levels of MPO nor improve histologic scores compared with controls
Results (after DSS challenge)	Reduced levels of MPO by 30%; reduced epithelial damage and lymphoid infiltration in the middle colon by at least 30%	Neither decrease levels of MPO nor improve histologic scores compared with controls

The above table shows that although the amount of TFF1 delivered by bacteria is more than 1500-fold less than the amount of purified TFF1 administered orally, the bacteria-delivered

mTFF1 showed surprisingly better results than orally administered TFF1 as reflected on the MPO levels and histologic scores (emphasis added). Clearly, delivery of mTFF for treatment and prevention of acute DSS-induced colitis was considerably more effective through in situ synthesis by *L. lactis* than through direct administration of purified mTFF to the intestinal tract.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Lothar Steidler

03/11/2005

Date November 13, 2005